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Published in:
Microbial Ecology

DOI:
10.1007/s00248-018-1213-1

Published: 01.01.2018

Document Version
Version created as part of publication process; publisher's layout; not normally made publicly available

Citation for pulished version (APA):
Väisänen, M., Gavazov, K., Krab, E. J., & Dorrepaal, E. (2018). The Legacy Effects of Winter Climate on Microbial Functioning After Snowmelt in a Subarctic Tundra. *Microbial Ecology*, *77*(1), 186-190. https://doi.org/10.1007/s00248-018-1213-1

The legacy effects of winter climate on microbial functioning after snowmelt in a subarctic tundra

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Abstract

Warming-induced increases in microbial CO_2 release in northern tundra may positively feedback to climate change. However, shifts in microbial extracellular enzyme activities (EEAs) may alter the impacts of warming over the longer-term. We investigated the *in situ* effects of 3-years of winter warming in combination with the *in vitro* effects of a rapid warming (6 days) on microbial CO_2 release and EEAs in a subarctic tundra heath after snowmelt in spring. Winter warming did not change microbial CO_2 release at ambient (10°C) or at rapidly increased temperatures, *i.e.* a warm spell (18°C) but induced changes ($P < 0.1$) in the Q_{10} of microbial respiration and an oxidative EEA. Thus, although warmer winters may induce legacy effects in microbial temperature acclimation, we found no evidence for changes in potential carbon mineralisation after spring thaw.

Keywords: snow manipulation, extracellular enzymes, β-glucosidase, phenol oxidase, microbial respiration, PLFA

The responses of soil microorganisms to climate warming control soil carbon releases and thereby determine the fate of the vast carbon stores in northern tundra soils in a warmer future [1, 2]. In tundra, winter warming is pronounced [3] and can directly enhance microbial activity during winter, whereas over the longer-term it can decrease ecosystem CO_2 emissions during summer [4, 5]. Warmer winters may enhance microbial carbon and nitrogen mineralization and thereby deplete labile soil carbon pools but also increase mineral nitrogen availability during the following summer [5–7]. Warming can also induce shifts in bacterial and fungal biomass thus altering the release of substrates from microbial necromass at snowmelt [7–9]. Soil substrate and nitrogen availability strongly control microbial communities and activities in tundra [10–12]. Therefore, although microbial communities turn over at snowmelt [13], the aforementioned changes during winter can carry-over to affect microbial summer community composition and activity, and consequently CO_2 releases from tundra soils. The mechanisms behind these legacy effects on microbial CO_2 release remain poorly understood.

Chronic warming often decreases microbial CO_2 release while increasing its temperature sensitivity (Q_{10}), possibly through depletion of labile substrates and a shift to increased decomposition of more complex substrates [14, 15]. Depolymerisation of these substrates requires more activation energy and is thus more temperature sensitive [16]. However, the substrate depolymerisation is not solely driven by temperature but also by microbes and their responses to temperature. Therefore, warming-induced changes in microbial community composition, carbon-use efficiency, and extracellular enzyme activities (EEAs) can control CO_2 release [17–21]. Since EEs catalyse the depolymerisation of organic substrates [20], warming-induced changes in EEs can be essential determinants of decomposition. Warming can increase microbial synthesis of EEs and thereby the maximum rates of EEAs enhancing decomposition; In addition, warming may induce microbial temperature acclimation that can alter microbial capacity to synthesize isozymes (*i.e.* EEs having similar functions but different temperature responses) thereby shifting the Q10 of EEAs [22].

In tundra, decomposition is slow and, consequently, the accumulated soil organic matter consists predominantly of plant originating complex compounds, such as lignin, ligno-cellulose complexes and celluloses [23–25]. Among EEs, phenol oxidase (PO) and β-glucosidase (BG) are important for soil carbon dynamics in tundra, since they catalyse the initial, possibly rate-limiting steps of breaking down the abundant lignin (PO) and cellulose (BG) polymers producing more labile products that can be easily mineralized [16]. It is unknown, however, whether preceding winter conditions can affect these EEs and their responses to temperature after snowmelt. Identifying such seasonal legacy interactions may increase our understanding of the integrated effects of gradual (seasons to years) climate warming and rapid (days) temperature increases on the EEAs that drive soil carbon losses.

To investigate the legacy of winter conditions on microbial CO_2 release, we collected soils directly after snowmelt from a subarctic, alpine cryoturbated tundra heath in northern Sweden (68°18.030′N, 19°7.262′E, 860 m a.s.l. [26]) where two types of warming manipulations had been conducted for three consecutive years. Gardening fleeces increased soil temperatures from autumn until mid-winter (0.6–1.0 K), whereas snow accumulation behind snow fences increased temperatures from autumn until mid-winter (0.4–1.6 K; Suppl. table 1, [27]). We measured microbial CO_2 release and Q_{10} from these soils during a shortterm laboratory incubation that included a six-day warm spell. To investigate the underlying mechanisms of microbial CO_2 release after snowmelt, we analysed fresh soils for fungal and bacterial biomass, and both fresh and incubated soils for their maximum activity and Q_{10} of EEs. We hypothesized that both field-warming treatments would decrease microbial CO_2 release and increase its Q_{10} in association with shifts in fungal: bacterial ratio, carbon-use efficiency and EEAs. We expected that this would be most pronounced in soils from the warmer snow fence treatment.

Soils were sampled on 3 June 2015 by taking 2–3 cores (diam. 4.8 cm) from the top (2–8 cm) organic soil horizon at each experimental plot (n=15). There were six replicates for control, six

replicates for fleece and three replicates for snow fence (Suppl. fig. 1). Soils were homogenized, and on 5 June, an incubation experiment was started, in which the homogenised subsamples (10 g fresh weight) were incubated at 10°C for 10 days and then, after water addition, at 18°C for six days to simulate a warm spell (Suppl. fig. 1). At our experimental site, daily maximum temperatures rise to 10°C soon after snow melt (Suppl. fig. 2) and, thereby, the incubation conditions during the first ten days resembled natural temperature regimes. During the incubation, microbial CO_2 release was measured repeatedly with an infrared gas analyser (EGM-4, PPSystems, USA) and standardized by soil organic matter (SOM) and time. The average respiration rates at 10°C and 18°C were used to calculate Q_{10} as follows:

$$Q_{10} = \left(\frac{CO2\_18}{CO2\_10}\right)^{\left(\frac{10}{18-10}\right)}$$

Both fresh and incubated soils were analysed for moisture (16 h, 105°C) and fresh soils for SOM content (4 h, 475°C, Table 1). Subsamples of fresh soils (4 g fresh weight) were frozen and analysed for PLFAs [28, 29] that were identified to bacterial and fungal markers [30–33] to get fungal: bacterial ratio and total microbial PLFAs that included also unidentified PLFA markers (Table 1). Microbial mass-specific respiration (a proxy of carbon-use efficiency [34]) was calculated by normalizing microbial CO_2 release with total PLFAs and time. The effects of winter warming on the activities of PO and BG at 10 and 18 °C were analysed both in fresh and in incubated soils. The EEAs were analysed from soil-phosphate buffer (0.1 M, pH 4.4; soil pH range at the site is 3.9–4.8) slurries mixed with chromogenic substrates (5 mM paranitrophenyl-β-glucopyranoside, BG; 5 mM L-DOPA, PO) [35–37]. After incubations at either 10°C (3.5 and 5 h for BG and PO, respectively), or 18°C (1 and 1.5 h for BG and PO, respectively), BG (405 nm) and PO (450 nm) activities were detected (Jasco V-650 spectrophotometer). Paranitrophenol (BG) and oxidation of L-DOPA with mushroom tyrosinase (PO) were used for extinction coefficients and EEAs were standardized by SOM and time.

We use the EEAs at 10 and 18 °C to assess their potential maximum activity and the Q_{10} calculated as [38]:

$$Q_{10} = e^{\left(10\times\left(\frac{LNEE18-LNEE10}{8}\right)\right)}$$

We use the shifts in the Q_{10} between fresh and incubated soils as an additional index of microbial temperature acclimation. Univariate statistics (linear mixed models) for microbial respiration and EEAs, biomass and fungal: bacterial ratios were conducted with IBM SPSS Statistics 24 (Suppl. Table 2).

Three consecutive years of winter warming had no impacts on microbial respiration after snowmelt (Fig. 1a, at 10°C). Accordingly, microbial mass-specific respiration (Fig. 1b, at 10°C), fungal: bacterial ratio (Table 1), and the maximum activities and Q_{10} of BG and PO in fresh soils (Fig. 1c, d, Day 0) were similar across the treatments. These findings are in accordance with a comparable study in a subarctic peatbog that reported of no changes in microbial community composition or EEAs after altered winter conditions [38, 39]. It therefore appears that changes of shorter duration in winter climate may not induce any changes in microbes under average late spring climate.

Alternatively, antecedent warmer winters may rather affect the response of the microbial activities to more extreme conditions, such as a warm spell, leading to cumulative effects on microbial functioning after a certain time lapse [5]. Indeed, after the lab-simulated warm spell, the Q_{10} of microbial respiration increased 15.3% in the snow fence treatment relative to the other treatments (Fig. 1a; effect of winter warming, $P = 0.061$). In addition, the Q_{10} of PO declined 59.2% by warming incubation in the control but remained constant in both winter warming treatments over the incubation (Fig. 1d; winter warming × incubation interaction, $P = 0.097$). Although we cannot exclude the possibility that these effects were overestimated because of the low number of replicates of the snow treatment and the natural spatial heterogeneity of tundra soils, they match previous

findings showing that in tundra, oxidative EEs respond to climate warming and have a high temperature sensitivity [40, 41].

The changes in the Q_{10} of respiration and PO activities after winter warming could be caused by changes in the availability of organic matter. However, although SOM content was rather variable at our study site, the SOM content did not differ consistently between the treatments (Table 1). Soil substrate availability is a strong environmental cue controlling microbial synthesis of EEs [42]. Since the treatments had no effects on the maximum rates of BG and PO, changes in the availability of chemically varying organic substrates, such as lignin and celluloses that are the substrates of BG and PO, do not seem a plausible reason. We suggest that the higher Q_{10} of microbial respiration and the altered Q_{10} of PO in response to the warm spell thus indicates microbial temperature acclimation [16, 22] due to warmer winter conditions.

Nevertheless, the treatment-specific shifts in the Q_{10} of microbial respiration and PO did not drive basal microbial respiration, which doubled due to the lab-simulated warm spell and was paralleled by a three-fold increase in the maximum activity of BG (at 18 °C) over the incubation across all treatments (Fig. 1a, c). The observed overall decline in PO activity after the imposed warm spell (Fig. 1d) likely resulted from our applied standardized water addition halfway the incubation period, as PO is sensitive to increases in soil moisture [20, 43]. However, this should not have resulted in treatment-specific effects on Q_{10}'s, as soil moisture contents did not differ between treatments (Table 1). In tundra, hydrolytic EEAs, such as BG, dominate in relatively wet spring soils, whereas oxidative EEs, such as PO, dominate during the dryer summer [40]. Thus, later in summer once hydrolytic activities decrease, winter warming-induced temperature acclimation in PO activity could potentially affect the rate limiting steps of lignin and ligno-cellulose decomposition.

To conclude, three years of winter warming did not affect springtime microbial carbon-mineralization or its underlying drivers, fungal: bacterial ratios, biomass-specific respiration and EEAs. However, our winter warming scenarios induced changes in the Q_{10}'s of microbial

respiration and PO activity possibly via microbial temperature acclimation. Although this microbial acclimation could carry-over to affect microbial activities during summer, our results indicate that winter warming does not affect microbial carbon mineralization at subarctic tundra heath after snowmelt under ambient or even under rapidly warmed conditions.

Acknowledgements

Jacob DeKraai assisted in field and laboratory work. PLFA analyses were conducted in the École Polytechnique Fédérale de Lausanne (EPFL), School of Architecture, Civil and Environmental Engineering (ENAC), Laboratory of Ecological Systems (ECOS), in Lausanne, Switzerland. Ann Milbau, Jonatan Klaminder, Frida Keuper, Kobayashi Makoto, Gesche Blume-Werry and Marina Becher assisted in setting the experimental design. This project was funded by grants from Vetenskapsrådet (621-2011-5444), Formas (214-2011-788) and a Wallenberg Academy Fellowship (KAW 2012.0152) to E. Dorrepaal.

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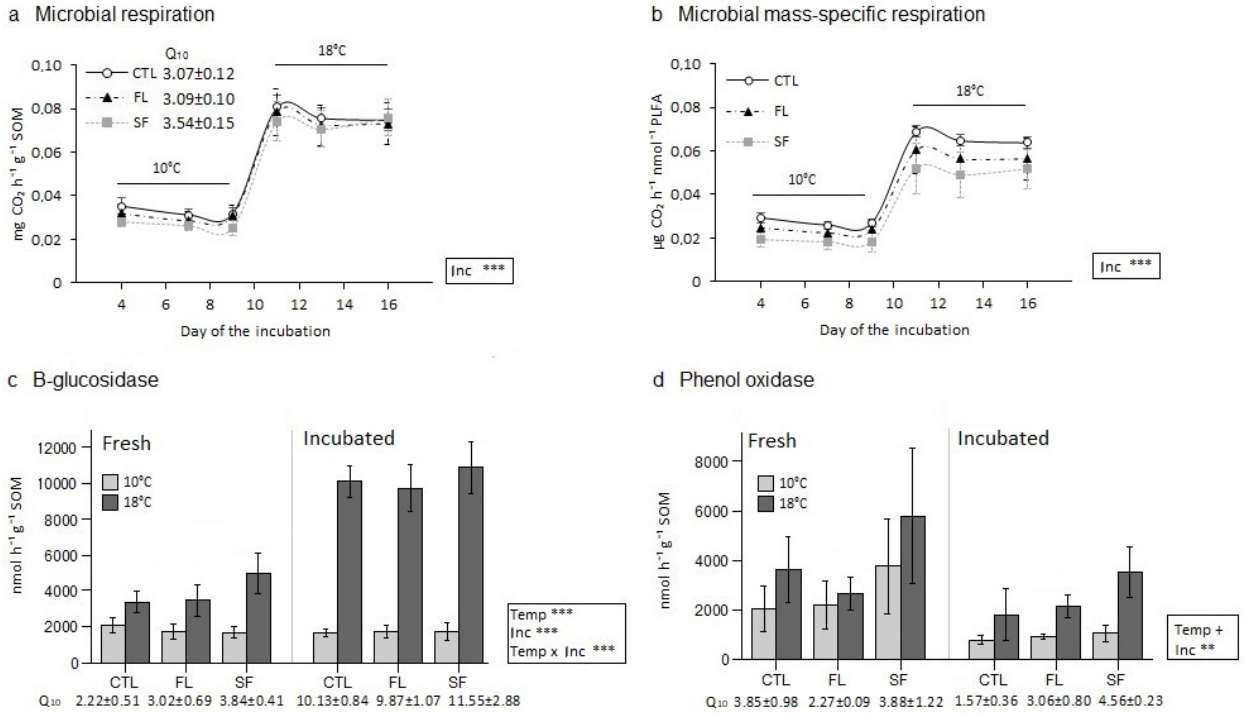
Figure legend

Figure 1 Microbial activities in soils exposed to 3 years *in situ* winter warming and a consequent short-term *in vitro* warming. Presented are treatment means and standard error bars for CTL = control (n = 6), FL = fleece insulation (n = 6), and SF = snow fence (n = 3). **a** Potential microbial respiration and its Q_{10} values and **b** microbial biomass-specific respiration were measured at six points during the short-term laboratory incubation. The incubation consisted of 10 days at 10°C, at day 8 water (2 mL) was added, and 6 days at 18°C. **c** Potential maximum activity of β-glucosidase (BG) and **d** phenol oxidase (PO) were measured at two points, in fresh soils before the incubation and after the incubation. At both occasions, potential maximum activity was assayed at 10°C and 18°C. Statistically significant (*** $P < 0.001$, ** $P < 0.01$, + $P < 0.1$) effects of incubation (Inc) and assay temperature (Temp) on respiration rates and EEAs are reported inside the small boxes. The Q_{10} of the EEA rates in fresh and incubated soils are reported below each treatment.

Table 1. Soil moisture, organic matter content (SOM), total microbial PLFAs, and fungal to bacterial PLFA (F: B) ratio analysed in fresh soils sampled right after snow melt (3 June 2015). The SOM content is shown for blocks 1–6 and, in addition, for control and fleece treatments in blocks 1–3 to increase comparability with snow fences that were only present in these three blocks. To control for the effects of water addition at day 8, soil moisture was analysed also after the short-term laboratory incubation. Treatment codes represent: CTL = control (n = 6), FL = fleece insulation (n = 6), SF = snow fence (n = 3). Values present mean (SE). There were no statistically significant differences between treatments in fresh soils. Accordingly, soil moisture did not differ between treatments in the incubated soils but was higher in comparison to fresh soils.

Soil variable	**CTL**	**FL**	**SF**
Moisture (g g^{-1} fresh soil)			
Fresh	0.45 (0.03)	0.40 (0.03)	0.34 (0.05)
Incubated	0.55 (0.03)	0.49 (0.02)	0.44 (0.04)
SOM (%)			
Blocks 1–6	24.4 (3.4)	20.2 (3.4)	15.4 (3.9)
Blocks 1–3	23.6 (7.2)	15.9 (3.5)	
Total PLFAs (nmol g^{-1} dry soil)	279 (30)	239 (18)	213 (7)
F: B ratio	0.68 (0.07)	0.69 (0.07)	0.55 (0.12)


a Microbial respiration
Q10
CTL 3.07±0.12
FL 3.09±0.10
SF 3.54±0.15
10°C
18°C
mg CO2 h-1 g-1 SOM
Day of the incubation
Inc ***
b Microbial mass-specific respiration
CTL
FL
SF
10°C
18°C
µg CO2 h-1 nmol-1 PLFA
Day of the incubation
Inc ***
c B-glucosidase
Fresh
Incubated
10°C
18°C
nmol h-1 g-1 SOM
CTL FL SF CTL FL SF
Q10 2.22±0.51 3.02±0.69 3.84±0.41 10.13±0.84 9.87±1.07 11.55±2.88
Temp ***
Inc ***
Temp x Inc ***
d Phenol oxidase
Fresh
Incubated
10°C
18°C
nmol h-1 g-1 SOM
CTL FL SF CTL FL SF
Q10 3.85±0.98 2.27±0.09 3.88±1.22 1.57±0.36 3.06±0.80 4.56±0.23
Temp +
Inc **

Supplementary table 1. Soil temperatures at the experimental site in northern Sweden for five different sub-seasons. Soil temperature at 1 cm depth was logged (Tiny Tag Talk 2, Intab Interface-Teknik AB, Sweden, n = 15) on hourly intervals during the period when the gardening fleeces were on (23 September 2014 – 17 May 2015) and during the late spring preceding soil sampling that was conducted shortly after snowmelt, 3 June 2015. Mean soil temperatures at top soil horizons down to 10 cm depth have been reported to be very similar [1]. Therefore, our soil temperature records from the 1 cm depth provide a reliable proxy of soil temperatures in the sampled, 2–8 cm thick top soil horizons. The average values for soil mean, maximum and minimum temperatures for each sub-season are reported. Treatment codes represent: CTL = control (n = 6), FL = fleece insulation (n = 6), SF = snow fence (n = 3). In the fleece treatment, snowmelt timing was equal to the control treatment, whereas in the snow fence treatment, snowmelt was delayed about a week. Significant ($P < 0.05$) differences between treatments within each sub-season based on Bonferroni *post hoc* tests are highlighted and indicated with different letters and marginally significant ($P < 0.10$) differences are pointed out with letters and +.

Sub-season	Period		CTL	FL	SF
Autumn	23 September – 31 October	*Mean*	**-0.76 (0.12) a**	**-0.15 (0.11) b**	**-0.41 (0.08) ab**
		Max	5.48 (0.48) a	6.47 (0.46) a	5.57 (0.12) a
		Min	**-4.32 (0.55) a+**	**-2.89 (0.21) b+**	**-3.33 (0.15) ab+**
Midwinter	1 November – 15 January	*Mean*	**-5.45 (0.35) a**	**-4.48 (0.41) ab**	**-3.82 (0.14) b**
		Max	**-0.97 (0.07) a**	**-0.63 (0.07) b**	**-0.64 (0.07) b**
		Min	**-12.5 (0.99) a+**	**-10.3 (0.97) b+**	**-9.56 (0.47) b+**
Late winter	16 January – 31 March	*Mean*	-6.63 (0.57) a	-6.31 (0.44) a	-5.29 (0.75) a
		Max	-2.69 (0.11) a	-2.86 (0.28) a	-2.87 (0.47) a
		Min	**-13.8 (1.09) a+**	**-12.1 (0.85) ab+**	**-9.70 (0.24) b+**
Spring	1 April – 17 May	*Mean*	-1.82 (0.24) a	-1.50 (0.27) a	-1.91 (0.37) a
		Max	10.1 (0.88) a	10.6 (2.58) a	10.1 (3.04) a
		Min	-5.89 (0.43) a	-5.81 (0.34) a	-4.63 (0.39) a
Late spring	18 May – 2 June	*Mean*	2.61 (0.26) a	2.88 (0.30) a	1.77 (0.49) a
		Max	12.7 (1.46) a	11.7 (0.96) a	12.5 (2.56) a
		Min	-0.80 (0.28) a	-0.26 (0.06) a	-0.46 (0.13) a

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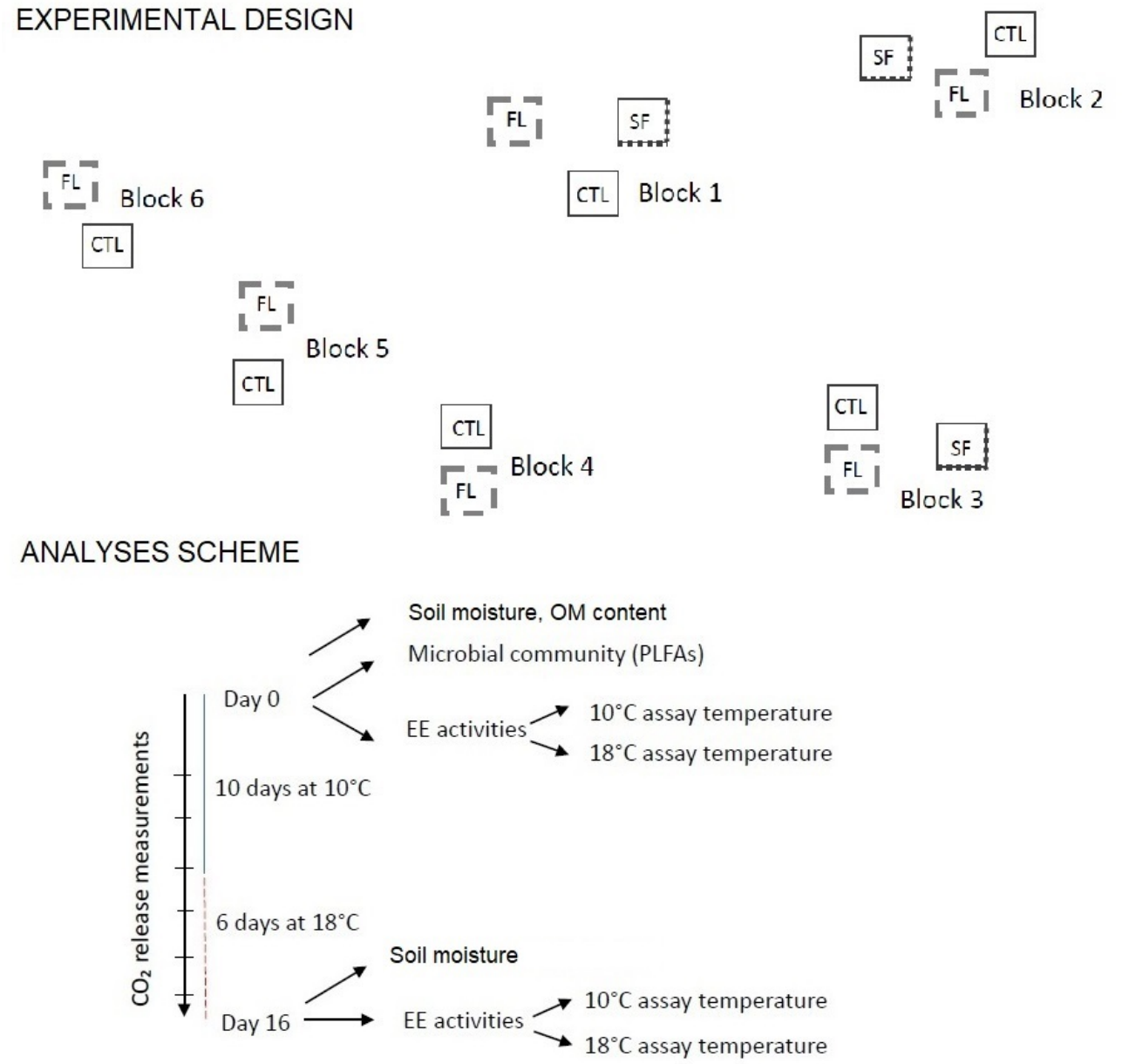
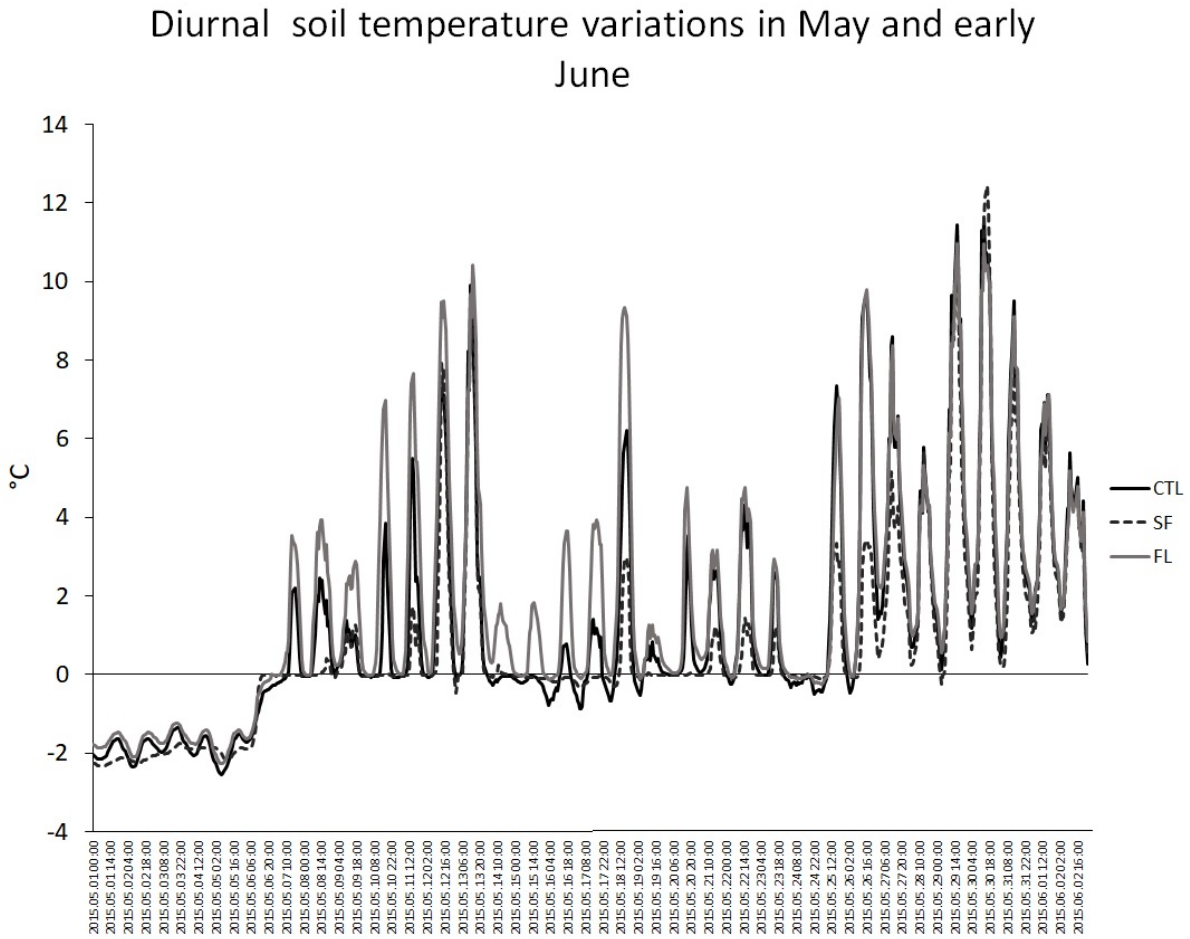


Supplementary figure 1. Experimental design and analyses scheme. The experiment was a block design that consisted of six blocks laid on an area of approx. 1.5 ha. All blocks had a control plot (CTL) and a plot that was warmed from autumn until late spring with insulating gardening fleeces (FL). In addition, blocks 1–3 had a plot with snow accumulating snow fence (SF) that insulated soil during the snow covered season. The distance between the experimental plots was 2–5 m within each block, whereas the distance between the blocks was always >10 m. In 3 June 2015, composite soil samples (2–3 cores, diam. 4.8 cm) were collected from each experimental plot from the top organic

horizon (2–8 cm depth) and stored in cool (+4°C). After sieving (2 mm mesh) and removal of stones and roots, in 5 June 2015, one set of sub-samples (4 g fresh weight) was immediately frozen (-20°C) and later on freeze-dried for the analyses of bacterial and fungal PLFAs. A second set of sub-samples (2 g fresh weight) was analysed for soil moisture and organic matter content, and a third sub-sample (6 g fresh weight) was analysed for the activities of extracellular enzymes at two different assay temperatures (10°C and 18°C). A fourth set of sub-samples (10 g fresh weight) was placed into incubation vials (235 mL) and the short-term incubation experiment was started. The soils were first incubated in darkness 10 days at 10°C and on day 8, 2 mL deionized water was added to avoid drying. The 10°C is the ambient mean summer soil temperature at the study site [1] and also corresponds to the daily peak soil temperatures at the study site in May and early June (Suppl. fig. 2). Microbial CO_2 release was measured two times before water addition and once after water addition. Then, incubation temperature was increased to 18°C and incubation continued for six days and CO_2 release was measured three times. The 18°C corresponds to the maximum summer soil temperatures at the study site (Väisänen M, unpublished data). After the incubation, the soil was analysed again for extracellular enzyme activities, and for soil moisture.

References

1. Krab EJ, Rönnefarth J, Becher M, Blume-Werry G, Keuper F, Klaminder J, Kreyling J, Makoto K, Milbau A, Dorrepaal E (2018) Winter warming effects on tundra shrub performance are species-specific and dependent on spring conditions. J Ecol 106:599–612



Supplementary figure 2. The diurnal soil temperatures in May and early June 2015 preceding the soil sampling (3 June) at the study site. Soil temperature was measured at 1 cm depth. Treatment codes represent: CTL = control (n = 6), SF = snow fence (n = 3), FL = fleece insulation (n = 6).

Supplementary table 2. The mixed model statistical designs used for analysing the effects of chronic winter and spring warming treatments, short-term laboratory incubation and assay temperature on measured variables. For warming treatments, the number of treatment levels was always three and for plot identity, always 15. Microbial respiration was analysed at six time points during the incubation, whereas β-glucosidase (BG), phenol oxidase (PO) and their Q_{10} values were analysed at two time points – in fresh soils before incubation and after the incubation. Logarithmic transformations were made to meet model assumptions of normality and homoscedasticity and model fits were assessed by residual plots and Akaike's Information Criteria.

Variable	*Fixed factors*	*Random factor*
Microbial respiration, mass-specific respiration	Incubation Winter warming	Plot identity
BG, PO	Incubation Winter warming Assay temperature	Plot identity
BG Q_{10}, PO Q_{10}	Incubation Winter warming	Plot identity
Respiration Q_{10} Soil moisture SOM content (%) Total PLFAs Fungal: bacterial ratio	Winter warming	
Soil mean, maximum and minimum temperature within each sub-season	Winter warming	